Remarks

Claims 40-45 and 48 are presently being examined. The present application is a US national phase application from PCT application PCT/US00/13292, which was properly filed within 12 months after the filing of the provisional application on May 12, 2000 and properly claimed priority to the provisional application as required under PCT rules. An amendment to the specification now establishes the priority date of the national filing under 35 USC §371. The request by the Examiner to elect a species for an amplifiable particle is traversed because the claimed composition is directed to a selenocystein-containing peptide fused to a surface protein displayed on an amplifiable genetic particle. The concept of an amplifiable genetic particle is a well-understood term of art and is addressed on page 9 of the response.

The Claimed Invention

Applicants respectfully submit that a brief explanation of the claimed invention will facilitate the progress of prosecution as it appears that there is some confusion relating to description and enablement requirements over what is new and what is well established in the art.

The claimed invention is directed to a novel composition that is an improvement over the prior art concerning fusion proteins displayed on the surface of amplifiable genetic particles. This novel composition is distinguished from fusion proteins of the prior art by the presence of

selenocysteine. Selenocysteine provides a unique reactive site on the displayed polypeptide. An advantage of this system over others is that the bacterial genetic machinery can be employed to incorporate the unique reactive site into the displayed peptide. This maintains the link between displayed peptide sequence and DNA sequence. The incorporation of selenocysteine into the fusion protein provides a novel combination of *in vivo* biomolecular amplification and unlimited diversity of small molecule chemistry (pg 4, line 11).

Indeed, the background to the claimed invention states in the first two lines that:

> The fusion of peptides to the coat protein of amplifiable genetic particles, eg phage, is a widely used method for screening combinatorial libraries of peptides. [citing references] (page 1, line 1 of the specification).

The background continues by describing how random peptide sequences can be expressed to form libraries with a complexity of 10^9 complexity.

The background also reports that:

> A vast body of work has been carried out with libraries consisting of systematic variations of peptides, ...peptide analogs ... and small molecules [citing references] (page 1, line 26)

In particular, the background cites enzymatically phosphorylated tyrosines and biotinylated lysine residues (page 3 of the application). Modifications such as these however are not encoded on the phage genome severing the link between displayed sequence and DNA sequence. The present claimed invention elegantly solves this problem. Because the displayed peptide sequence is encoded by DNA in the amplifiable particle, the single peptide can be cloned and

recovered from a library in which binding of the ligand to a peptide on the amplifiable particle is a one-in-millions event. Without the ability to clone and recover the peptide to which the ligand binds, recovery of the peptide would be problematic.

Rejection under 35 U.S.C. § 112 first paragraph

The Examiner has objected to the following terms as lacking written description.

(a) In claim 40, the term "covalently linked" has been substituted by the term "fused" and the phrase "positioned on an amplifiable particle" has been substituted by the phrase "displayed on the surface of a genetically amplifiable particle". Support for these amendments can be found for example in the abstract, and pages 10, 16 and 17 of the application.

(b) In amended claim 41, "the amplifiable genetic particle is selected from a phage, a virus, a cell or a spore."

The definition of an amplifiable genetic particle as a phage, cell, spore or virus is accepted by those of ordinary skill in the art. For example, this definition is provided in US 5,223,409 issued by the United States Patent and Trademark Office to Dyax in 1993 (see column 7, line 59, and claim 1), the term "genetic package" being interchangeable with "genetic particle" (see the use of "particle" throughout the text, for example, col 60, line 46, col 85, line 24). The expression of fusion proteins on the surface of genetic particles is

established not only by the above-cited patent but also in numerous other references. For example: Jostock et al. (2005) Comb. Chem. High Throughput Screen (8) pp 127-33 reports that

> "A multitude of systems for the presentation of foreign peptides or proteins on the surface of microorganisms has been developed within the last two decades" (see attached abstracts)

A brief scan of Medline reveals many references including the attached abstracts confirming the diversity of amplifiable genetic particles used for peptide display in the prior art.

Wernerus et al. Biotechnology Appl. Biochem (2004) vol 40, pp 209-28 reports on the display of peptides on the surface of gram positive bacteria (spores) and gram negative bacterial cells. Kieke et al. PNAS (1999) vol 96, pp 5651 and Boder et al. Nature Biotechnology (1997) vol 15, pp553-7 report on the expression of fusion proteins on the cell surface of yeast cells. The above references are representative of the widespread use of cells, spores and viruses with known surface proteins capable of displaying surface protein fusions on their surface. Applicants assert that no undue experimentation is required to include a selenocysteine insertion sequence into a DNA within these amplifiable genetic particles in order to express a selenocysteine containing peptide fused to a surface protein.

(c) The phrase in claim 42 has been amended so that the phrase "located at a predetermined unique site" has been substituted by the phrase "incorporated at a specific unique site". Support for this amendment can be found on page 4, lines 12-14.

(d) The term "a native peptide bond" has been substituted by the term "peptide bond". The term "native" is redundant as a peptide bond is understood in the art to be an amide linkage between two amino acids (also see Grant and Hackh's chemical dictionary (5th Edition 1987 pub.McGraw-Hill).

(e) The phrase "part or all" of a selenocysteine insertion sequence has been deleted. A selenocysteine insertion sequence is described in Figures 1, 2 and 4. Figure 1 shows how a selenocysteine amino acid is incorporated during expression of a peptide in response to a selenocysteine insertion sequence (SECIS). Figure 2 shows in detail the components of a selenocysteine insertion sequence which includes: an invariant loop; a stem structure containing an invariant bulge; an 11 nucleotide spacing which can be unpaired; and a UGA codon. Figure 4 shows a selenocysteine insertion sequence in a nucleotide sequence encoding a fusion protein, for example, the selenocysteine peptide fused to a surface protein that is the subject of claim 40.

(f) The phrase "located adjacent to one or more nucleotides" has been substituted by the phrase "downstream from the TGA codon" Support for this phrase can be found in figures 1, 2 and 4.

(g) Support for the fusion of a selenocysteine peptide fused to surface proteins displayed on an amplifiable genetic particle is discussed above in (b). Applicants would like to emphasize again that fusion of a peptide to a surface protein on an amplifiable particle has been described extensively in the prior art for a wide variety of

genetically amplifiable particles. The claimed inventive improvement is the inclusion of a selenocysteine codon within a peptide or library of peptides. The selenocysteine-containing peptide is thus a target for chemical modification of the displayed peptide and thus introduces the ability to tether virtually any desired chemical functionality to the incorporated selenocysteine for the first time (see page 4).

On page 7 of the office action dated 2/17/05, the Examiner has asserted that the claimed fusion protein lacks a fixed amino acid structure but "merely requires the presence of selenocysteines" Applicants assert that the presence of the selenocysteine in the peptide is a primary focus of the claimed invention. This is not a functional description as implied by the Examiner. It is a structural feature and distinguishes the claimed composition from the prior art. Hence the ruling in University of California v. Eli Lilly is not applicable.

Furthermore, the application clearly states (see for example, the background) and it is well known to a person of ordinary skill in the art that an important function of a peptide fused to a surface protein on an amplifiable genetic particle is for purposes of screening libraries of peptides for their ability to bind a particular ligand. This is a well-established approach and a person of ordinary skill in the art could make such a library without undue experimentation. There would be no purpose nor would it be feasible to describe the peptide structure of each of 10^9 peptides typically cloned in a library of peptide fusion proteins. However, should one of ordinary skill in the art following the teaching of the present claimed invention wish to insert a selenocysteine into the peptide or peptide library, they could readily

follow the detailed description of how to achieve this provided in the specification.

The Examiner asserts that a person of ordinary skill in the art would not distinguish the claimed subject matter because there is no description of the size, configuration or other structural feature of the peptide portion, the surface protein or the amplifiable particle.

Applicants assert that one of ordinary skill in the art would recognize what is meant by an amplifiable genetic particle and a surface protein. The abstracts attached to this response are a sample that illustrates the extensive prior art concerning the use of fusion proteins comprising a peptide (lacking a selenocysteine) fused to a surface protein displayed on the surface of an amplifiable genetic particle.

Rejection under 35 U.S.C. § 112 second paragraph

(a) The Examiner has again objected to "positioned on an amplifiable particle" in claim 40. Claim 40 has been amended as discussed above so that this rejection is now believed to be moot.

(b) The Examiner has objected to "amplifiable particle" in claim 40. Claim 40 has been amended to utilize the term in the abstract and throughout, namely, "amplifiable genetic particle". The meaning in this context would seem unambigious. Since the advent of DNA amplification through PCR, amplification has come to mean multiplication. Amplifiable genetic particle is thus understood to mean

multiplication of particles that contain DNA such that the particles may be cloned. Clonability is generally associated with the use of such particles. (see page 1 of the application.). This is generally applicable to viruses, spores and cells.

(c) The Examiner has again objected to "located at a predetermined unique site" in claim 42. The claim has been amended and the objection is now believed to be moot.

(d) The Examiner has again objected to "native peptide bond" in claim 43. The claim has been amended and the objection is now believed to be moot.

(e) The Examiner has objected to the term "adjacent" in claim 45. The claim has been amended to specify directionality. A common characteristic of the selenocysteine insertion sequence is that it is required to occur downstream of the TGA.

(f) The Examiner has objected to the Markush group of claim 48. The members of the Markush group are all cells. The claim has been amended accordingly.

Rejection of the claims under 35 USC §§102(a) (b) and (e) and 103

The Examiner has cited Sandman et al JACS Feb 2000 and Sandman et al NAR (2000) under 35 USC 102(a) and (b). Both these references were published by present Applicants after the filing date of

the provisional application from which the present application gains priority and therefore do not constitute prior art.

The Examiner has cited Larson et al. 5,272,078 alone or in combination with Holliger as anticipating or rendering obvious claim 40. Claim 40 of the above application requires:

> A fusion protein comprising a selenocysteine-containing peptide fused to a surface protein displayed on an amplifiable genetic particle.

Larson

The field of the Larson reference is stated to be:

> The present invention relates to the cloning, characterization and uses of both human and rat Type I iodothyronine 5' deiodinase, a selenocysteine-containing enzyme involved in the conversion of thyroxine to 3,3',5-triiodothyronine. The invention further relates to one or more mutant forms of the enzyme and the use of genes coding for such mutant forms as reporter genes.

The Larson reference uses DNA from M13 phage as a vector only "Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage". According to the reference, the selenocysteine-containing protein appears to be expressed in eukaryotic or prokaryotic cells according to the desired post translational properties and the recombinant protein is then recovered from the cells.

The reference states

> An example of a terminal insertion includes a fusion of a signal sequence, whether heterologous or homologous to the host cell, to the N-terminus of the 5' deiodinase molecule to facilitate the

secretion of mature 5' deiodinase molecule from recombinant hosts.

The reference requires secretion. There is no suggestion that the selenocysteine-containing protein be expressed on the surface of phage M13 or indeed any other virus, cell or spore. In fact, Applicants searched the text of the patent for the word "surface" and no hit was found. Applicants assert that there is no motivation to combine this reference with the cited Holliger reference.

Applicants were unable to identify in the reference any suggestion or teaching that the recombinant protein prepared by Larson should be retained on the surface of an amplifiable particle such as M13 phage or that the recombinant protein be a fusion protein as required in claim 40 of the above application.

In summary, Applicants respectfully assert that the Larson reference refers to a separate and distinct invention and has no relevance to the present claimed invention. In particular, Applicants were unable to find support for the Examiner's assertion that the reference described that selenopeptides could be recombinantly expressed in M13 phage.

Holliger

This reference describes the solution structure of the g3P, a minor coat protein from filamentous phage fd. Although g3P is used in phage display libraries there is nothing in this reference that discusses

types of peptides fused to the g3P and nothing that suggests that selenocysteine be incorporated into a peptide fused to g3P.

The reference states in the last sentence of the introduction on page 4 that " The structure has allowed a meaningful sequence comparison with relevant domains from other filamentous phages indicating the presence of similar membrane-penetration domains."

This supports the fact that surface proteins in a variety of amplifiable genetic particles were well known in the art at the time of the claimed invention. The reference comments on peptide and protein fusions with g3P but suggests that it is the g3P itself can bind ligand.

Applicants respectfully submit that the references do not describe the present claimed invention either inherently or explicitly and request that the Examiner reverse the rejection.

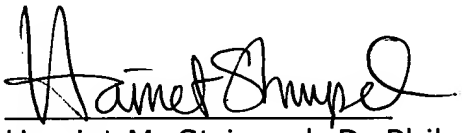
Summary

For the reasons set forth above, Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time to file a response and enclose a check in the amount of $510. Please charge Deposit Account No. 14-0740 for any deficiencies.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.



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Boder ET, Wittrup KD; *Nat Biotechnol.* 1997 Jun; 15(6):553-7.

Yeast surface display for screening combinatorial polypeptide libraries

Abstract

Display on the yeast cell wall is well suited for engineering mammalian cell-surface and secreted proteins (e.g., antibodies, receptors, cytokines) that require endoplasmic reticulum-specific post-translational processing for efficient folding and activity. C-terminal fusion to the Aga2p mating adhesion receptor of Saccharomyces cerevisiae has been used for the selection of scFv antibody fragments with threefold decreased antigen dissociation rate from a randomly mutated library. A eukaryotic host should alleviate expression biases present in bacterially propagated combinatorial libraries. Quantitative flow cytometric analysis enables fine discrimination of kinetic parameters for protein binding to soluble ligands.

Kieke et al.; *Proc Natl Acad Sci U S A.* 1999 May 11;96(10):5651-6.

Selection of functional T cell receptor mutants from a yeast surface-display library

Abstract

The heterodimeric alphabeta T cell receptor (TCR) for antigen is the key determinant of T cell specificity. The structure of the TCR is very similar to that of antibodies, but the engineering of TCRs by directed evolution with combinatorial display libraries has not been accomplished to date. Here, we report that yeast surface display of a TCR was achieved only after the mutation of specific variable region residues. These residues are located in two regions of the TCR, at the interface of the alpha- and beta-chains and in the beta-chain framework region that is thought to be in proximity to the CD3 signal-transduction complex. The mutations are encoded naturally in many antibody variable regions, indicating specific functional differences that have not been appreciated between TCRs and antibodies. The identification of these residues provides an explanation for the inherent difficulties in the display of wild-type TCRs compared with antibodies. Yeast-displayed mutant TCRs bind specifically to the peptide/MHC antigen, enabling engineering of soluble T cell receptors as specific T cell antagonists. This strategy of random mutagenesis followed by selection for surface expression may be of general use in the directed evolution of other eukaryotic proteins that are refractory to display.

Werneurs, H. and Stahl, S.; *Biotechnol Appl Biochem.* 2004 Dec; 40(Pt 3):209-28.

Biotechnological applications for surface-engineered bacteria

Abstract

Display of heterologous proteins on the surface of micro-organisms, enabled by means of recombinant DNA technology, has become an increasingly popular strategy in microbiology, biotechnology and vaccinology. Both Gram-negative and Gram-positive bacteria have been investigated for potential applications. The present review will describe the most commonly used systems for bacterial display, with a focus on the biotechnology applications. Live bacterial vaccine-delivery vehicles have long been investigated through the surface display of foreign antigens and, recently, 'second-generation' vaccine-delivery vehicles have been generated by the addition of mucosal targeting signals, as a means to increase immune responses. Engineered bacteria have also the potential to act as novel microbial biocatalysts with heterologous enzymes immobilized as surface exposed on the bacterial cell surface. They provide the potential for new types of whole-cell diagnostic devices, since single-chain antibodies and other type of tailor-made binding proteins can be displayed on bacteria. Bacteria with increased binding capacity for certain metal ions can be created, and potential environmental or biosensor applications for such recombinant bacteria as biosorbents are being explored. Certain bacteria have also been employed to display various polypeptide libraries for use as devices in in vitro selection applications. Part of the present review has been devoted to a more in-depth description of a promising Gram-positive display system, i.e. Staphylococcus carnosus, and its applications. The review describes the basic principles of the different bacterial display systems and discusses current uses and possible future trends of these emerging technologies.